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FIGURES

Fig. 1.

A

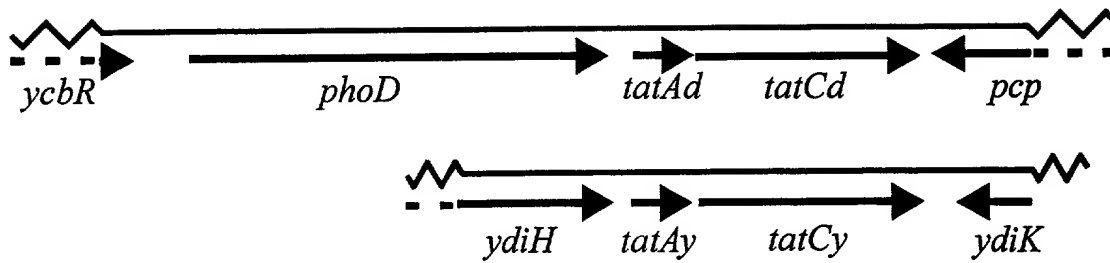
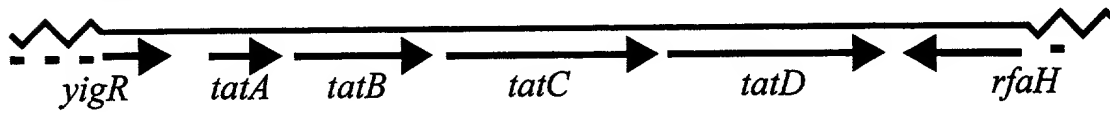
TatA (Eco)	M- SGISIWQLHTAVLVVLEFGSTKKLG -----	26
TatE (Eco)	M-GEISITIKLLVVAALVVILFSGTKKLR-----	26
TatAy (Bsu)	M--PIGPGSLAVIAIVAHILFQPKKL P-----	25
TatAd (Bsu)	MFSNIGT PGLIILIFVIALTFEGPSKLP -----	27
TatAc (Bsu)	M--ELSEFKILVILFVGFLVTGPDKLP -----	25
TatB (Eco)	MT-DIGFSELLVFILGLVALGPQRPLPVAVKTVAGWIRALRSLATTVQNELTQELKIQ	49
	* * *	
TatA (Eco)	-----SIGSDLGASIKGFKKAMSDDE---PKQDKTSQDADFTAKTI	64
TatE (Eco)	-----TLGGDLGAAIKGFFKAMNDDD---A-AAKKGADVLDLQAEKL	63
TatAy (Bsu)	-----ELGKAAGDTLREFKNATKGLT---SDEEEKKEDQ-----	57
TatAd (Bsu)	-----EIGRAAKRTLLEFKSATKSLV---SGDEKEEKSAELTAVK-	64
TatAc (Bsu)	-----ALGRAAGKALSEFKQATSGLT---QDIRKNDSSEN-----K-	57
TatB (Eco)	EFQDSLKKVEKASLTNLTPELKASMDRELQAESMKRSYVANDPEKASDEAHTIHNP	114
 * 	
TatA (Eco)	ADKQADTNQE-----QAKTEDAKRHDKSEQV	89
TatE (Eco)	SHKE-----	67
TatAy (Bsu)	-----	57
TatAd (Bsu)	-----QDKNAG	70
TatAc (Bsu)	-----EDKQM-	62
TatB (Eco)	VVKDNEAAHEGVTPAAAOQTQASSPEQKPETTPBPVVKPAADAEPKTAAPSPSSDDKP	171

B

TatC (Eco)	MSVEDTQ--PLITHLIELRKRLLENCITAVIVTELCVLYFANDIYH-LVSAPLIK	51
TatCy (Bsu)	MTRMKVNQMSLLEHIAELRKRLLIVALAFVVETLAGFFFLAKPIIVVLQETDEAK	50
TatCd (Bsu)	MDKKETH---LIGHLEELRRRLTVTLAAPELGGTAELGVDIDYDWLRDLDGK	51
	* . . . * . . . * . . . * . . . * . . . *	
TatC (Eco)	QLPQGSTMIA TDVASPFFTP I K L T F M V S I L L S A R V L L Y Q W A F I A P A L Y K H E R R	105
TatCy (Bsu)	QL----TLNAFNLTDPLEYVFMPQFAFLIGIVLTSPVILYLQWAFVSPGLYEKERK	104
TatCd (Bsu)	-----LAVLGPSSELVWVMMLSGICATAASTPAAYQLWRFWAPALTKTERK	98
 * * * * *	
TatC (Eco)	LVPVPLL---SSSLLEVTGMAFAYEVVVEPLAAGFLANTAPE-GVQVSTDIASLV	155
TatCy (Bsu)	VTLSYI---PVSIILLPLAAGLSRSFYVILRPVVDPMKRISQDLNVNQVIGINERYF	155
TatCd (Bsu)	VTIMYIMYIPLEALEFLAGISFGYFVLPEDVLSFLTHLSSG-HFETMFTADRYF	151
 * * * * * *	
TatC (Eco)	SFYMALFMAGVYSFEVPVAIVLLCWMGITSPEDLRKKRPLVVGAFVYGULLTE	209
TatCy (Bsu)	HFLLOLTIFPGLLFQMPEVILMPLTRLGVTPMFLAKIRKAFTPTLVITAALITE	209
TatCd (Bsu)	RFMNINSLRGPIFEMPELVMMFLTRLGILNPYRLAKARKSYELLQWSLNLTP	205
	* . . . * . . . * . . . * . . . * . . . * . . . *	
TatC (Eco)	PDVFSQTLLAIIPMYCLFETGVFFSRE-VGKGGRNREEENDAEASEKTEE	258
TatCy (Bsu)	PELLSHMMVTVPLLLIYEYSILSKAAYRKAQKSSAADRDVSSG-----Q	254
TatCd (Bsu)	PDFISDFLVMIPLLLYLEYSVTLSAFYKKRMRE-----ETAAA-----A	245
	* . . . * . . . * . . . * . . . * . . . *	

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Fig. 2.

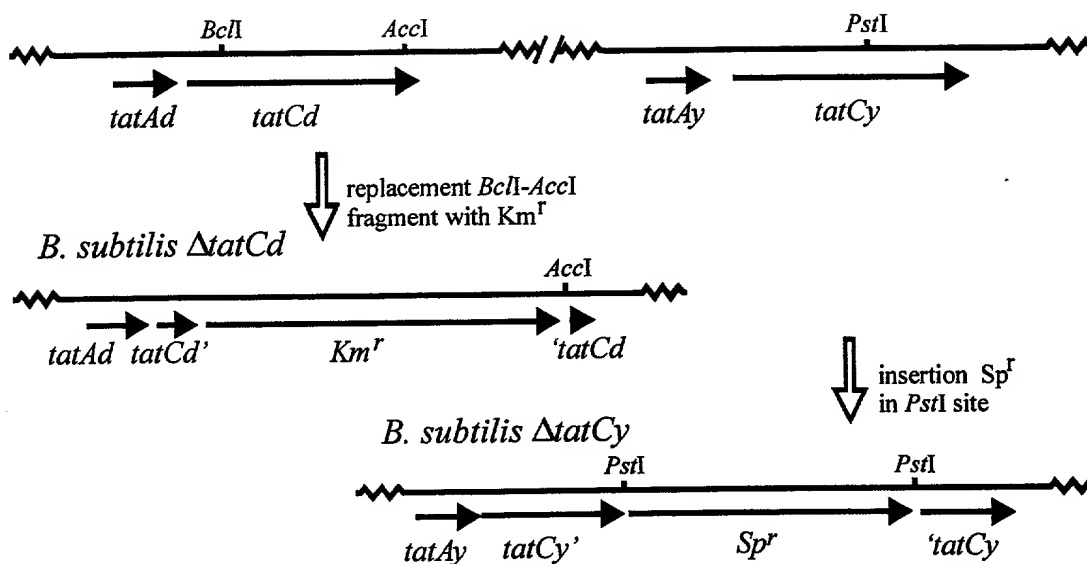
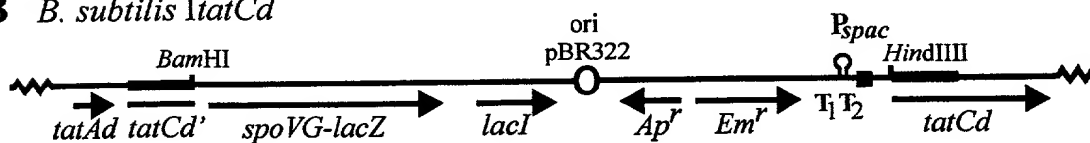
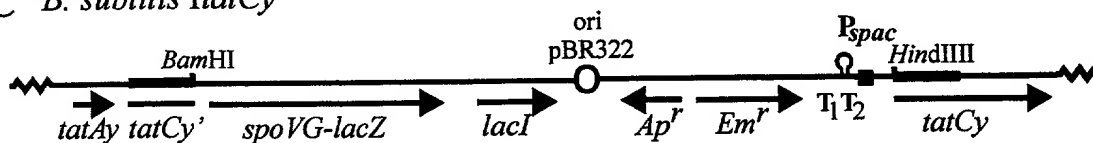
A *B. subtilis*B *E. coli*

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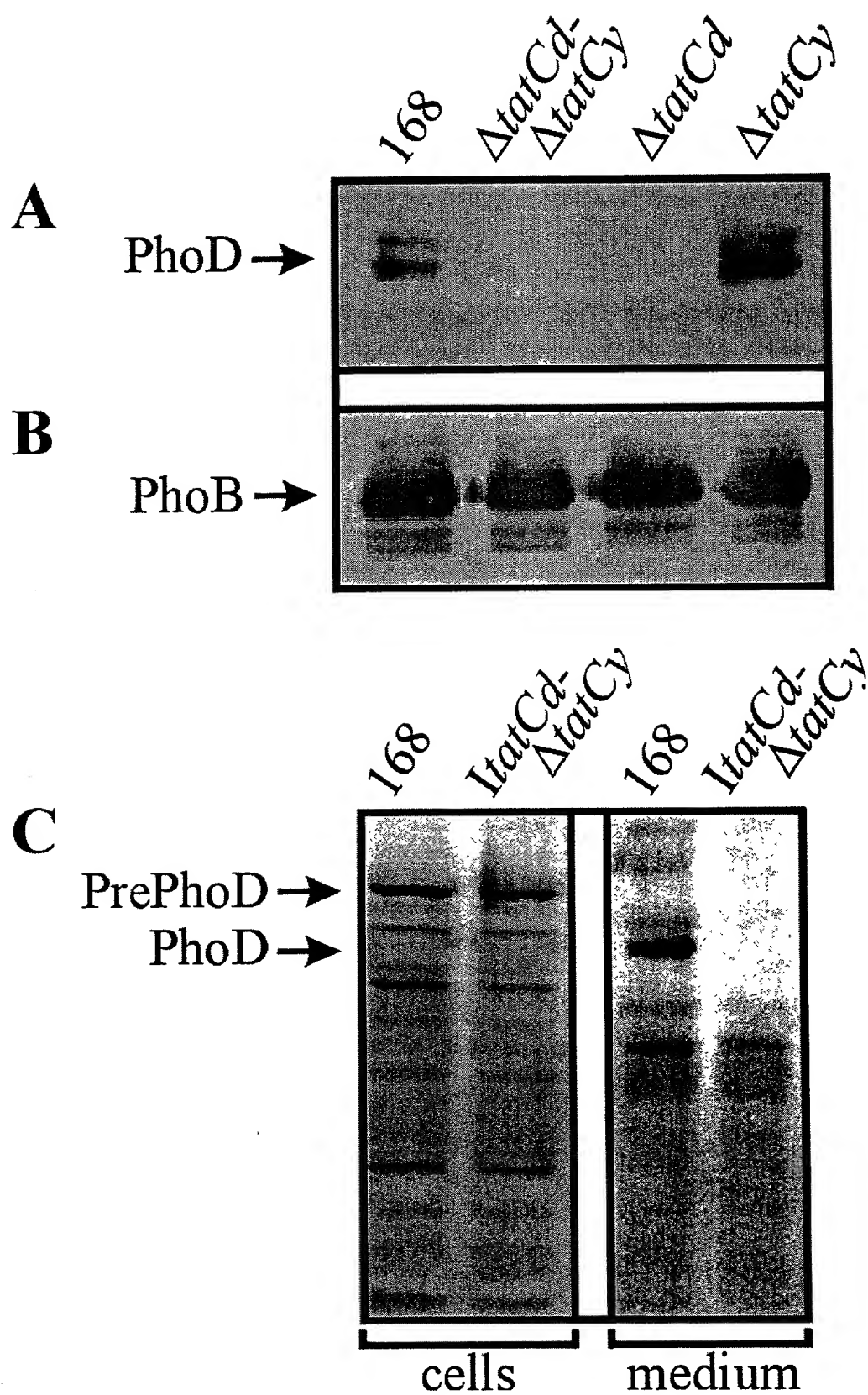
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Fig. 3.

A *B. subtilis* 168B *B. subtilis* $\Delta tatCd$ C *B. subtilis* $\Delta tatCy$ 

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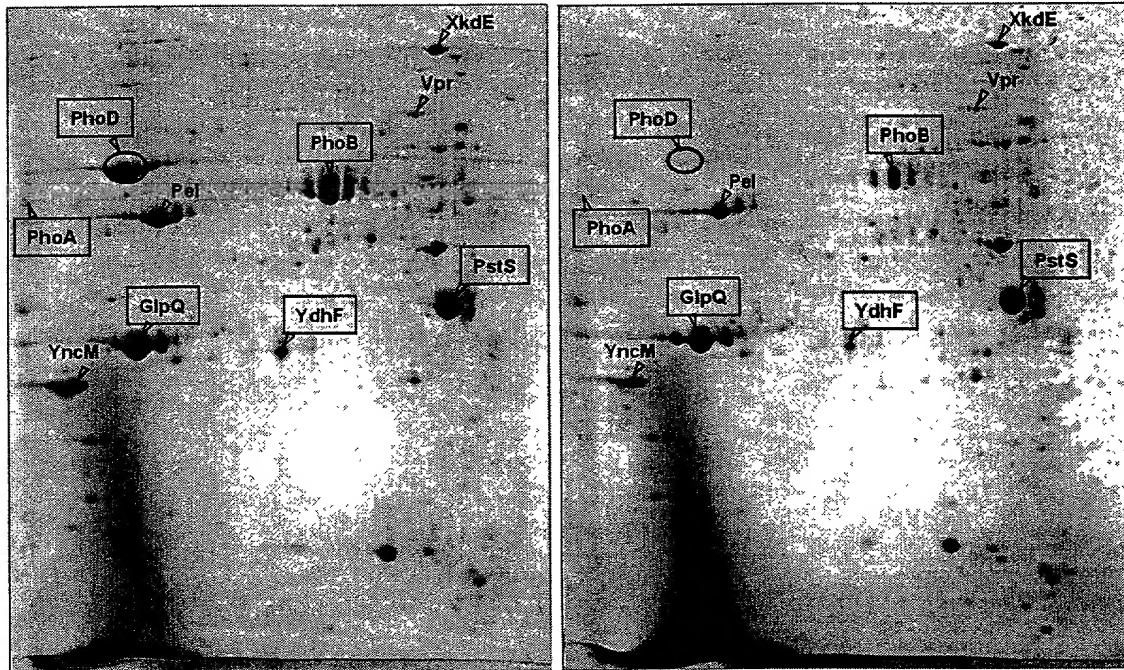
Fig. 4.



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Fig. 5.

168

 $\Delta tatCd-\Delta tatCy$ 

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FIGURE 6



Tat-dependent secretion of the *B. subtilis* lipase LipA. *B. subtilis* 168 (parental strain), *B. subtilis* ΔtatCd, *B. subtilis* ΔtatCy, or *B. subtilis* ΔtatCd-ΔtatCy were grown in TY-medium to end-exponential growth phase. To study the secretion of LipA, *B. subtilis* cells were separated from the growth medium by centrifugation. Proteins in the growth medium were concentrated 20-fold upon precipitation with trichloroacetic acid, and samples for polyacrylamide gel electrophoresis (SDS-PAGE) were prepared. Secreted LipA in the growth medium was visualized by SDS-PAGE and Western blotting, using LipA-specific antibodies.

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FIGURE 7

Predicted twin-arginine (RR-)signal peptides of *B. subtilis*¹

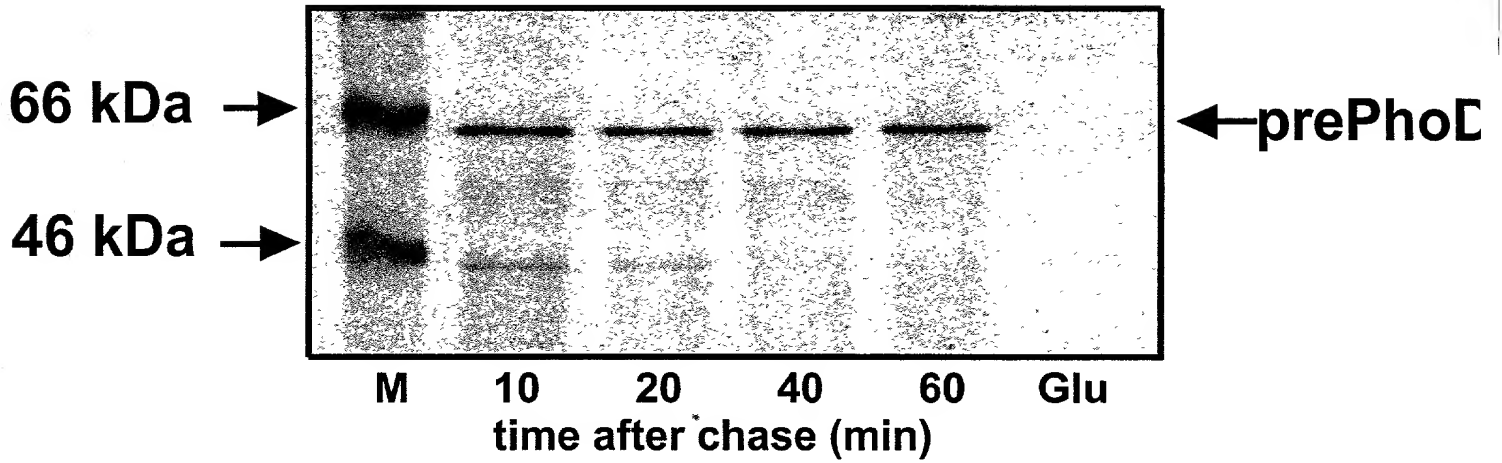
Protein	N	h	RR-Motif	H	h	C
AlbB	1	0.1	RRILL	27	2.0	AIA
AmyX TM	9	-0.8	RRSFE	15	1.1	-
AppB TM	8	0.5	RRTLm	19	2.3	-
LipA	7	-1.1	RRIIA	19	1.2	AKA
OppB TM	8	-0.6	RRLVY	24	2.0	-
PbpX	2	-2.2	RRRKL	14	2.9	WNA
PhoD	3	-1.3	RRKFI	17	0.9	VGA
QcrA TM	1	-1.1	RRQFL	19	1.3	-
TlpA TM	1	-0.8	RRLII	21	2.4	-
WapA ^W	1	-3.0	RRNFK	18	2.3	VLA
WprA	8	-1.7	RRKFS	20	1.9	AAA
YceA TM	1	-0.4	RR AFL	21	2.2	-
YesM TM	1	-1.5	RRMKI	20	2.4	QYA
YesW	1	-1.3	RRSCL	19	2.0	VKA
YfkN TM	1	-1.2	RRTHV	17	1.7	IHA
YkpC	8	-1.0	RRVAI	17	2.3	SLA
YkuE	1	-1.3	RRQFL	17	1.0	GYA
YmaC	7	0.0	RRFLL	15	2.4	YSL
YubF TM	9	-2.7	RRNTV	23	2.0	-
YuiC	8	0.2	RRLLM	20	1.9	IEA
YvhJ TM	2	-1.7	RRKIL	18	2.5	-
YwbN	1	-1.8	RRDIL	23	1.4	QTA

¹ The listed signal peptides contain, in addition to the twin-arginines, at least one other residue of the consensus sequence (R-R-X- ϕ - ϕ ; printed in bold). The number of residues in the N- and H-domains of each signal peptide, and the average hydrophobicity (h) of each of these domains, as determined by the algorithms of Kyte and Doolittle (Kyte, J., and R. F. Doolittle [1982] A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-32), are indicated. Furthermore, the RR-motifs in the N-domain, and SPase I recognition sites in the C-domain (*ie.* positions -3 to -1 relative to the predicted SPase cleavage site) are shown. Proteins lacking a (putative) SPase I cleavage site, some of which contain additional transmembrane domains, are indicated with "TM". One protein containing cell wall binding repeats is indicated with "W".

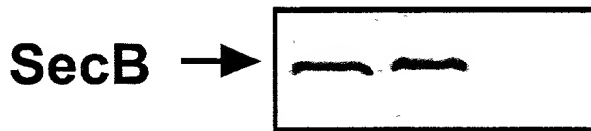
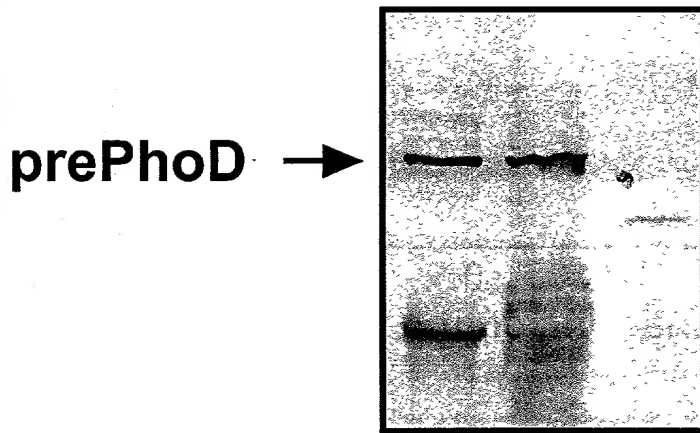
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A



B

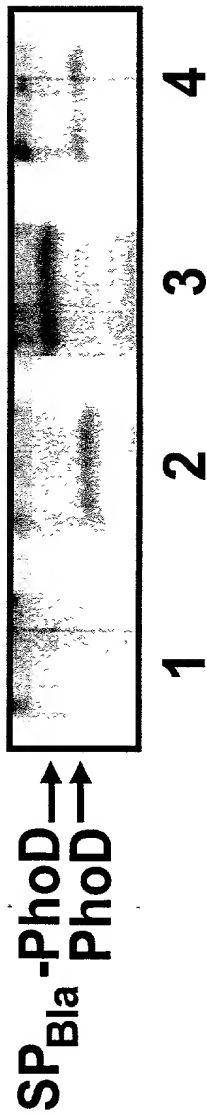


Proteinase K	-	+	+
Triton X-100	-	-	+

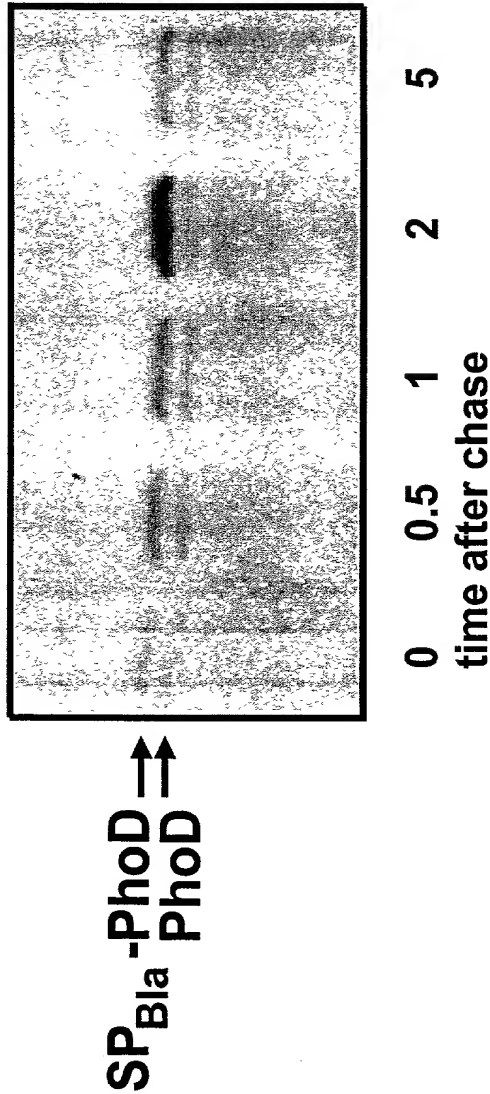
Figure **8**

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A



B untreated



C + NaN₃

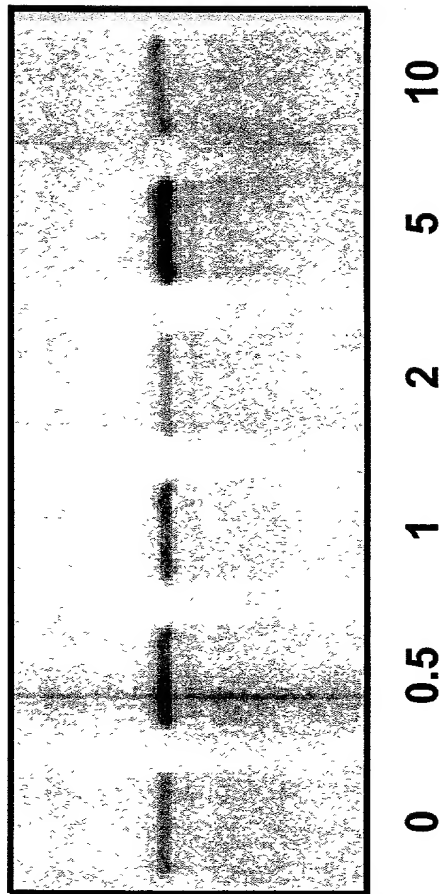
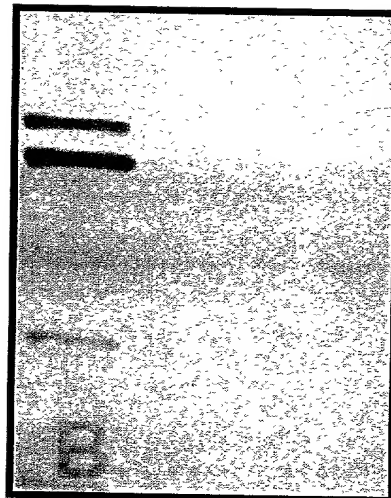
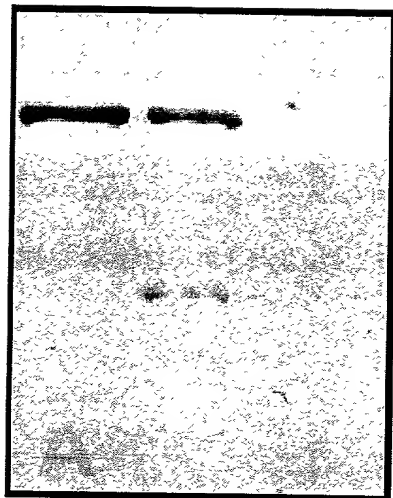


Figure 9

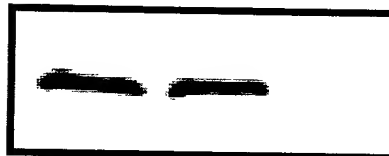
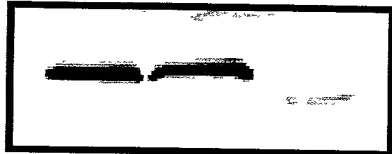
A

B

SP_{PhoD}-LacZ
LacZ



SecB



Proteinase K
Triton X-100

- + +
- - +

- + +
- - +

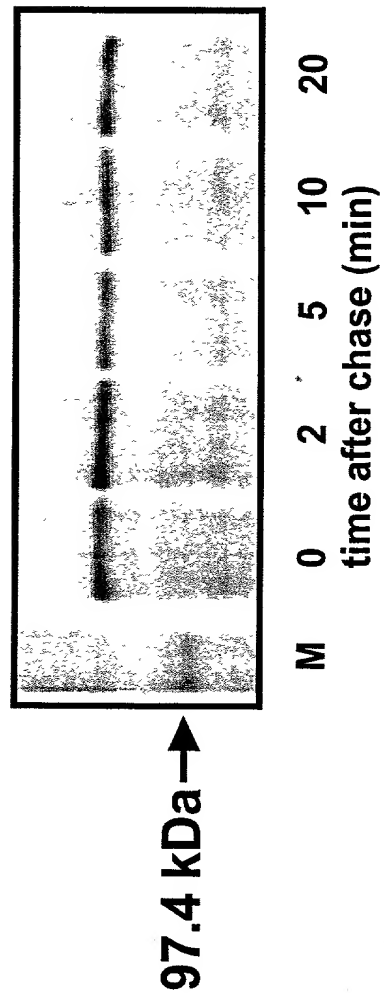
Figure 10

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TD2T60" 2E213650

A



B

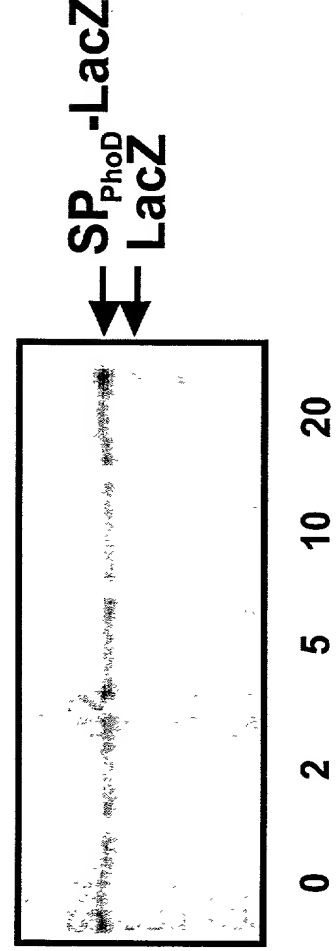


Figure 11

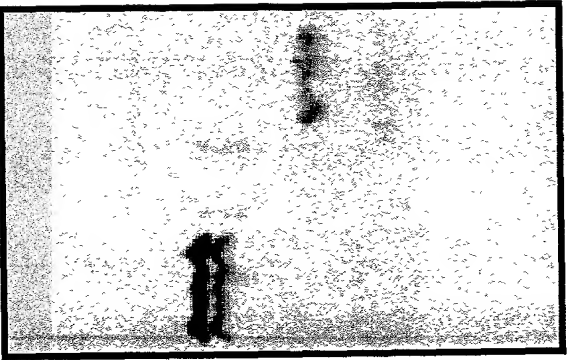
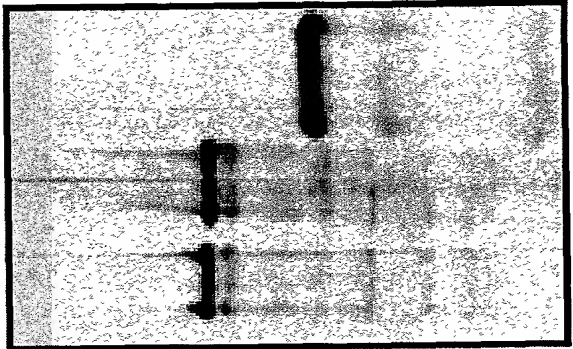
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A
+nigericin

B
+NaN₃

SP_{PhoD}-LacZ
LacZ

SecB

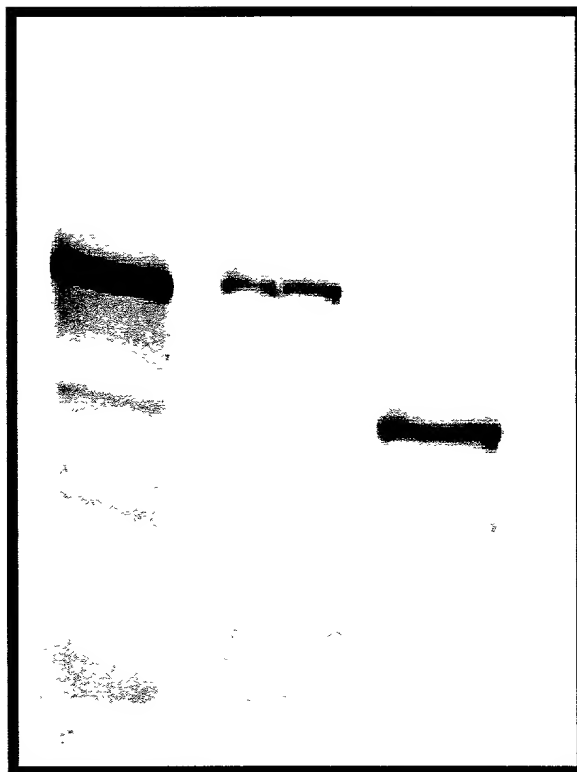


Proteinase K	-	+	+	-	+
Triton X-100	-	-	+	-	+

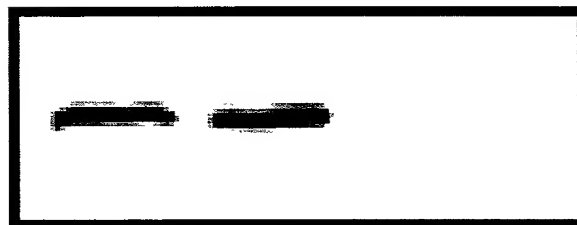
Figure 12

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SP_{PhoD}-LacZ →



SecB →



Proteinase K
Triton X-100

-	+	+
-	-	+

Figure 13

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Figure 14
Homologs in *B. alcalophilus*

TatA

**MGGLSVGSVVLIALVALLIFGPKKLPELGKAAGSTLREFKNATK
GLADDDDDTKSTNVQKEKA**

TatC

**MTMMTPNQQTSKKKKRKGRKGRVPMQDMSIMDHAEELRRRIF
VVLAFFIVALIGGFFLAVPVITFLQNSPQAADMPFNAFRLTDPLRV
YMNFAVITALVLIIPVILYQLWAFVSPGLKENEQKATLAYIPIAFL
LFLAGIAFSYFILLPFVISFMGQMADRLEINEMYGINEYFSFLFQL
TIPFGLLFQLPVVVMFLTRLGVVTPFTFLRKIRKYAYFALLVIAGII
TPPELTSHLFVTVPMLILYEISITISAITYRKYHGTTHNGQESAK**

FD-503 (Rev. 11-27-60)